

BIOASSAY, CARCINOGENESIS and TISSUE CULTURE

#521

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Application For Research Grant

Date: January 17, 1966

1. Name of Investigator: CESARE BIANCIFIORI, M.D.

2. Title: Division of Cancer Research

3. Institution & Address: University of Perugia
P.O. Box 167
Perugia, Italy

4. Project or Subject: Lung tumorigenesis by isoniazid (INH), its metabolite hydrazine sulphate (h.s.) and derivatives of hydrazine.

New potential carcinogens for the human lung (INH and h.s.) have been demonstrated in previous experiments on mice. The aims of this research project are:

- I. To study whether the carcinogenic action of INH and h.s. will take place in species of laboratory animals other than mice; and
- II. To study whether other derivatives of hydrazine are carcinogenic for mice.

5. Detailed Plan of Procedure (Use additional pages if more space is required.)

I. INH, a widely-used drug in the treatment of tuberculosis, and hydrazine, its principal metabolite, have been shown to be carcinogenic in various substrains of mice (J. Juhasz et al.: Ztschr. Krebsforsch. 62, 186-196, 1957; L. Severi: Nature 192, 217-218, 1961; Cancer Bull. 16, 79, 1964; C. Biancifiiori et al.: Nature 194, 488-489, 1962; Lav. Anat. Pat. Perugia 23, 115-128, 1963; ibidem 23, 209-220, 1963; Brit. J. Cancer 18, 543-550, 1964).

Recent experiments have shown that h.s. is carcinogenic also for rats (C. Biancifiiori et al.: in press), while C57BL/Cb/Se substrain mice have been shown to be resistant to the carcinogenic action of INH and of h.s. (unpublished data).

In order to study whether the carcinogenic action of INH and h.s. (its metabolite) will take place in species of laboratory animals other than mice, the following experiments will be carried out.

A. Carcinogenic action of INH and h.s. in rats.

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(5. IA continued)

Tests. 300 rats:

- 100 (50 m. and 50 f.) treated with INH and vitamin B₆;
- 100 (50 m. and 50 f.) treated with h.s.;
- 100 (50 m. and 50 f.) untreated as controls.

Treatment. INH: Each rat, at 8 weeks of age, will receive 1.0 ml of 2.0 per cent aqueous solution daily by stomach tube, for 24 weeks. A sufficient amount of vitamin B₆ will be added so as to prevent the polyneuritis frequently caused by this chemical and in this way avoid a high early-death rate among the animals due to this complaint.

h.s.: each rat, at 8 weeks of age, will receive 1.0 ml of 1.13 per cent aqueous solution daily by stomach tube, for 24 weeks.

Examination. All rats will be allowed to live for about 80 weeks, when they will be killed for examination. The lungs, liver, adrenals, ovaries and all the organs suspected of tumours will be removed for histological examination. The lungs will be examined with the aid of special technical procedures.

B. Carcinogenic action of INH and h.s. in golden hamsters.

Tests. 300 golden hamsters:

- 100 (50 m. and 50 f.) treated with INH and vitamin B₆;
- 100 (50 m. and 50 f.) treated with h.s.;
- 100 (50 m. and 50 f.) untreated as controls.

Treatment. INH and h.s. as in IA. The dose will be varied in relation to the weight of the animals.

Examination. As in IA.

C. Carcinogenic action of INH and h.s. in rabbits.

Tests. 120 rabbits:

- 40 (20 m. and 20 f.) treated with INH and vitamin B₆;
- 40 (20 m. and 20 f.) treated with h.s.;
- 40 (20 m. and 20 f.) untreated as controls.

Treatment. INH and h.s. as in IA. The dose will be varied in relation to the weight of the animals.

Examination. All rabbits will be allowed to live for about 104 weeks, when they will be killed for examination. The same organs as in IA will be removed for histological examination.

II. D.B. Clayson et al. (International Conference on Lung Tumours in Animals, Perugia, 24th to 29th June, 1965) have shown that some

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(5. II continued)

derivatives of hydrazine are carcinogenic for BALB/c/Cb/Se substrain mice. Benzoyl hydrazide, 2-methoxybenzoyl hydrazide and 4-methoxybenzoyl hydrazide increased the incidence of lung tumours; iproniazid and phenylhydrazine hydrochloride were the least effective.

In order to study whether other derivatives of hydrazine are carcinogenic for mice, the following experiments will be carried out. In these experiments BALB/c/Cb/Se and C57/Cb/Se substrain mice, susceptible and resistant respectively to the induction of lung tumours by INH and h.s., will be used.

A. Carcinogenic action of P nitro benzoyl hydrazide.

Tests. 200 BALB/c/Cb/Se substrain mice:

100 (50 m. and 50 f.) treated;

100 (50 m. and 50 f.) untreated as controls;

200 C57BL/Cb/Se substrain mice:

100 (50 m. and 50 f.) treated;

100 (50 m. and 50 f.) untreated as controls.

Treatment. The drug will be administered six times weekly in aqueous solution by stomach tube. The size of the daily dose will be determined from the results of preliminary toxicity tests. The treatment will be continued for 20 weeks.

Examination. All mice will be allowed to live for as long as possible and will be examined at their natural death. An untreated mouse of equivalent age will be killed whenever a treated mouse dies, in order to match the experimental and untreated groups as closely as possible. The lungs, liver, adrenals, ovaries and all the organs suspected of tumour will be removed for histological examination. The lung will be examined using special technical procedures.

B. Carcinogenic action of phenyl ethyl hydrazine sulphate.

Tests. 200 BALB/c/Cb/Se substrain mice:

100 (50 m. and 50 f.) treated;

100 (50 m. and 50 f.) untreated as controls;

200 C57BL/Cb/Se substrain mice:

100 (50 m. and 50 f.) treated;

100 (50 m. and 50 f.) untreated as controls.

Treatment and Examination. As in IIA.

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6. Budget Plan: (Per year)

	a. Salaries	\$ 4,300.00
	b. Expendable Supplies	2,900.00
	c. Other Expenses	300.00
(* 700 animal cages and 10 racks for cages: \$2,500.00)	d. Permanent Equipment *	2,500.00
	e. Overhead (15% of a, b, c)	1,300.00
	Total	11,300.00

7. Anticipated Duration of Work: A period of three years will be necessary for the completion of the project - from March 1, 1966 to February 28, 1969.

8. Facilities and Staff Available: All the facilities existing at the Division of Cancer Research are available for this research project. The Division occupies two buildings, one of three floors and the other of four. The animal centre includes 32 rooms; mouse-rooms, storage-rooms, cold-rooms, fully-equipped food production unit and various services. In the animal centre (14) inbred substrains of mice, 3 of rats, 1 of golden hamsters and 1 of rabbits are maintained.

The Division also includes fully-equipped laboratories for histology (6 rooms), histochemistry and cytology (6 rooms), virology and tissue culture (13 rooms), biochemistry (9 rooms), library and offices for investigators. Of the staff employed in the Division, one histological technician and one animal caretaker will be available full

9. Additional Requirements: time for this project.

NIL

10. Additional Information (including relation of work to other projects and other sources of support):

The demonstration that INH and h.s. induce pulmonary tumours in mice is of extreme theoretical importance insofar as human pathology is concerned, and it must be developed step by step through the study of the carcinogenic action of these two chemicals in laboratory animals other than mice. Experiments along these lines are an absolute requirement at the present time in view of the progressive increase in lung tumours in human beings and of the widespread therapeutic and prophylactic use made of INH in human tuberculosis.

Collateral studies with hydrazine derivatives have the aim of testing their carcinogenic action and of analyzing more closely the mechanism of action of INH and hydrazine as carcinogenic agents in living hosts.

Leonora Biancifiore

Signature: DR. CESARE BIANCIFIORI, M.D.

Director of Project

Prof. Lucio Severi, M.D. *Lucio Severi*

Director, Division of Cancer Research,
Bioscience Resource Institute
Perugia, Italy.

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CURRICULUM VITAE

Dr. Cesare Biancifiore, Senior Assistant, Division of Cancer Research,
University of Perugia. **REDACTED**, Italy.

Present Citizenship: Italian. Sex: Male.

a. Education experience:

Degree	Institution conferring	Fields	Year
Baccalaur.	Liceo Classico "G. Tacito" Terni	---	1939
M.D.	University of Perugia	Medicine	1945
Specialist	University of Rome	Resp. Dis.	1959
L.D.	Ministry of Public Instruction	Morbid Anat.	1956

b. Other research training and experience:

Institution	Nature	Year
Institute of Morbid Anatomy and Division of Cancer Research, Perugia	Cancer research in animals and in human beings	1953/1965
Chest Hosp. INPS Perugia	Resp. dis. and tuberc.	1946/1965

c. Fields of present major scientific interest:

Carcinogenic action of INH and related compounds in laboratory animals. Cancer of the lung in human beings, respiratory diseases and tuberculosis.

d. Supplementary information:

Dr. Biancifiore has published 65 papers on various subjects, the most recent of them regarding the carcinogenic action of INH in mice. He has visited several institutions for Cancer Research in Holland and in Great Britain.

Since 1959, Dr. Biancifiore has been acting vice-director of the Chest Hospital, I.N.P.S. Perugia. He has worked with Dr. G.M. Bonser in the Department of Experimental Pathology and Cancer Research, School of Medicine, Leeds, and continues to co-operate with her, and now, also, with Dr. D.B. Clayson of the same department in Leeds. In collaboration with Dr. Bonser he has published various papers on mammary cancer induced by chemical carcinogens in mice under different hormonal status.

Since 1959 Dr. Biancifiore has been studying the carcinogenic action of INH, hydrazine and its derivatives. Several papers have already been published on this problem and other will be in the near future. In some of these papers Dr. D.B. Clayson was a co-author, and he is still collaborating.

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PAPERS FOR WHICH REPRINTS ARE NOT YET
AVAILABLE

1. Histogenesis of pulmonary tumours by hydrazine sulphate in BALB/c/Cb/Se substrain mice. (In collaboration with F.E. Giornelli-Santilli, U. Milia and E. Bucciarelli)
Presented at:
International Conference on Lung Tumours in Animals. Division of Cancer Research, University of Perugia, 24th to 29th June, 1965.
In press in the Proceedings of the Conference.
2. The induction of pulmonary tumours in BALB/c/Cb/Se mice by derivatives of hydrazine. (In collaboration with D.B. Clayson, U. Milia and F.E. Giornelli-Santilli)
Presented at:
International Conference on Lung Tumours in Animals. Division of Cancer Research, University of Perugia, 24th to 29th June, 1965.
In press in the Proceedings of the Conference.
3. Pulmonary carcinogenesis by (oral and intraperitoneal) administration of hydrazine sulphate in newborn BALB/c/Cb/Se substrain mice. (In collaboration with U. Milia and F.E. Giornelli-Santilli)
Presented at:
6th Annual General Meeting of British Association for Cancer Research, Trinity College, Dublin, September 23-25, 1965.
In press in "Lav. Anat. Pat. Perugia".
4. Pulmonary tumours in rats by oral hydrazine sulphate. (In collaboration with F.E. Giornelli-Santilli, U. Milia and L. Severi)
Submitted for publication to "Nature".

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